



Evaluation of Total Antioxidant and Oxidant Status, Oxidative Stress Index and DPPH Free Radical Scavenging Activities of Pumpkin (*Cucurbita maxima*) Seeds Ethanolic Extracts[#]

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ARTICLE INFO

[#]This study was presented at the 6th International Anatolian Agriculture, Food, Environment and Biology Congress (Kütahya, TARGID 2022)

Research Article

Received : 19/10/2022

Accepted : 24/11/2022

Keywords:

Pumpkin
Seeds
Antioxidant
Extracts
Free radicals

ABSTRACT

Fruits and vegetables are basic crops of mass consumption for human population to meet their food requirements. Recent advances in medicinal studies have revealed that different parts of fruits and vegetables are loaded with phytochemicals responsible for eliminating the risks of different diseases. Pumpkin (*Cucurbita maxima* Duchesne) is one of the abundantly grown and consumed vegetable all around the world. Seeds of this vegetables are named as nutritional power houses due to their excellent nutrient profiles. In present study total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI) and DPPH free radical scavenging activity, of pumpkin seeds extracts were determined. Extraction of pumpkin seeds was carried out in a Soxhlet apparatus using ethanol as extraction solvent. TAS and TOS were determined through Rel Assay Kits and free radical scavenging activity was measured through DPPH assay. TAS was found 4.18 ± 0.36 and TOS 14.68 ± 0.42 , whereas value of OSI was measured 0.35 ± 0.10 . DPPH free radical scavenging activity was observed increasing with increasing the concentration and maximum value $56.10 \pm 0.90\%$ was observed at 2 mg/mL concentration. From these results it was concluded that pumpkin seeds could be utilized as natural antioxidant source with potential to reduce oxidative stress and minimizing the risks of certain diseases.

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Introduction

Fruits and vegetables parts (peels, flesh, seeds) have been found involved in reducing the risks of many diseases due to presence of phytochemicals, responsible for different health promoting activities in humans. Pumpkin seeds due to presence of phenolic compounds, minerals, vitamins, carotenoids and essential oils, possess strong antioxidant potential and reduce oxidative stress in human body (Hussain et al., 2022). Extracts of different herbs, fruits and vegetables have been found loaded with biologically active components and are a well alternative source of drugs. Due to insufficient health services in many developing and under develop countries the need of complimentary medicines has been increased (Mohammed

et al., 2020). Expectations of public are very high from food producers in terms of healthy and nutritious food products, which can play both functional and medicinal role in the body and most economic and extraordinary sources of functional ingredients are waste by products of fruits and vegetables (Hussain et al., 2022). Plants are natural sources of antioxidant compounds and extracts of these plants and their parts have been traditionally used in curing different ailments due to their antioxidant, antimicrobial, antiviral, anti-inflammatory, antiallergic, antihypertensive and anticarcinogenic properties (Uysal et al., 2021).

Pumpkins are the members of family *Cucurbitaceae* and genus *Cucurbita* and their usage as a traditional food treating diseases is very common since ancient times. The excellent pharmacological properties of pumpkins are due to their phytochemical composition, which includes phenols, flavonoids, carotenoids and tocopherols. Among pumpkin parts, seeds are the most suitable component with respect to their chemical composition and their usage in different food products in different forms has gained very importance in recent times (Hussain et al., 2022). Production of pumpkins, squashes and gourds in 2019 was estimated above 23 million tons cultivated all over the world comprising an area of 1.54 million hectare and in Pakistan 2.7 lac tons on 26515-hectare area (FAO, 2019).

Plants especially fruits and vegetables produce secondary metabolites due to different metabolic reaction in their parts and these non-nutritive components have been reported involved in a range of biological activities in human bodies, when consumed in fresh or processed forms. Specially the antioxidant properties of different plant species and their parts like leaves, peels and seeds, are edge in lowering disease factors (Kina et al., 2021). Consumption of pumpkin seeds on a regular basis could be beneficial for both healthy and diseased persons, due to the excellent nutritional profiles of these seeds. Nutrients present in pumpkin seeds play mediating role in oxidation related reactions resulting in the protection of body cells. So many biologically active components have been reported in pumpkin seeds, which are capable of scavenging free radicals in the human body (Hussain et al., 2021). Ingredients used in the development of so many drugs drive their source from plants, fungi and animals. A variety of natural ingredients from extracts of plants are used in complimentary medicines in different regions of the world. Discovery of new plants, analysis of their different parts and extraction of active compounds is the need of time to overcome the need of modern drugs (Akgul et al., 2022).

Oils extracted from raw pumpkin seeds due to its antioxidant potential, significantly reduced serum triglycerides, total cholesterol, LDL cholesterol, uric acid, creatinine, serum transaminases and urea in experimental animals, whereas serum HDL cholesterol were significantly increased, which was a clear indication of health promoting potential of pumpkin seeds (Nameni et al., 2021). Investigations made by Majid et al. (2020) on humans by application of pumpkin seed oil, in controlled lifestyle, showed a significant reduction in endpoint LDL, significant increase in HDL cholesterol. Experiment results revealed pumpkin seed oil have potential of hypolipidemic and anti-hypertensive activity as it lowered LDL and increased HDL levels. Pumpkin seed oil extracts exhibited anti-oxidant, anti-bacterial and anti-inflammatory activities therefore, pumpkin seed oil could be a promising source of ingredients for pharmaceutical and food industries to develop pharma foods and supplements (Amin et al., 2020). Experiments were conducted to check the antioxidant status of pumpkin seeds and results revealed that pumpkin seed oil retarded liver damage by inhibiting oxidative stress induced by sodium nitrate in adult rats (Rouag et al., 2020). As pumpkin seeds are excellent source of nutrients that's why these are known as nutritional powerhouse. Pumpkin seeds have no side

effects on human health so these can be consumed on regular basis to promote human health with minimum use of supplements (Maheshwari et al., 2015). A recent study by Hussain et al. (2022), provided different utilization patterns of nutritious pumpkin, which included fresh, boiled, cooked, powders, extracts, isolates, purified bioactives and pumpkin based functional food products. Healthy eating, comprised of balanced diet equipped with pumpkin components could protect the body cells and systems from the attack of novel, viral and deadly diseases.

In this context it is very important to investigate peels, flesh and seeds of different medicinal plants, especially fruits and vegetables for their health promoting activities. Antioxidant properties of different plant portions and the agents responsible for this are needed to be discovered for benefit of mankind. Present study was designed to investigate the extracts of pumpkin seeds for their total antioxidant status, total oxidant status, oxidative stress index and DPPH free radical scavenging activity in comparison with ascorbic acid.

Materials and Methods

Procurement and preparations of plant materials

Mature pumpkins were collected from the fields directly and specimen was submitted to Botanical research Centre for identification. Seeds were separated from pumpkins after cutting with knife. Seeds were cleaned from any adhering fibrous material. Seeds were dried in hot air microwave oven at 60°C till constant weight. Fine powder was obtained after grinding the dried seeds in common spice grinder.

Preparations of pumpkin seeds extracts

For preparation of pumpkin seeds extracts, 20 g of powder was taken in flask and 200 mL ethanol was added and extraction was carried out at 50°C for approximately 6 hours. The solvents of the prepared extracts were removed in the rotary evaporator.

Determination of total antioxidant status, oxidant status and oxidative stress index

Total antioxidant status (TAS) and total oxidant status (TOS) of pumpkin seeds ethanolic extracts were measured using Rel assay kits by following the guidelines provided by Erel (2005) with some modifications. Trolox was used as standard calibrator in determination of TAS, whereas hydrogen peroxide was used as standard calibrator in TOS analysis. Oxidative stress index (OSI) was calculated as proportion of TOS and TAS values as described by Sevindik (2019).

Determination of DPPH free radical scavenging activity

For determination of DPPH free radical scavenging activity of pumpkin seeds ethanolic extracts at different concentrations, stock solutions of extracts were prepared using dimethyl sulfoxide at 0.25, 0.5, 1 and 2 mg/mL concentrations. DPPH solution (0.039%) was taken 160 µL in a cuvette and 50 µL stock solution was added and mixture was incubated for 30 minutes before analysis and then absorbance was measured at 517 nm using spectrophotometer. Same process was repeatedly carried

out for all selected concentrations of pumpkin seeds extracts by following the guidelines given by Shimada et al. (1992). Standard reference antioxidant in this study was used ascorbic acid.

Results And Discussion

Antioxidant status, oxidant status and oxidative stress index of pumpkin seeds extracts

Results of TAS, TOS and OSI have been presented in Table 1, from where it can be seen that TAS of pumpkin seeds ethanolic extracts was found 4.18 ± 0.36 mmol/L and TOS was found 14.68 ± 0.42 μ mol/L, which means that pumpkin seeds possess more antioxidant potential. OSI was calculated as ratio between TAS and TOS and value was noted as 0.35 ± 0.10 .

Production of free radicals in human body by different enzymatic and non-enzymatic processes is very common, which is caused by different factors including environmental and internal body reactions. As a result of these free radicals, oxidative stress is produced in the body cells and this oxidative stress results in advancement and occurrence of so many diseases including cancer, brain and heart diseases (Sevindik et al., 2021). Natural body defense system works to reduce oxidative stress caused by free radicals, but in many cases this natural defense system does not work sufficient to protect body cells. Supplementary antioxidant agents from different plant sources are then added to complimentary medicines and foods to support scavenging of free radicals (Mohammed et al., 2021). Antioxidant and oxidant status of a medicinal plant *Echium italicum* was determined by Uysal et al. (2021), and supportive results were found in terms of antioxidant capacity of plants and their parts. Similarly, Mohammed et al. (2020) collected plants from different regions of Iraq and determined their antioxidant status, oxidant status and oxidative stress index, and it was concluded that ethanolic extracts of plants have high antioxidant and low oxidant potential so these plants and their parts in different forms can be used as natural antioxidant agents. Recently a bioactive component of isoflavones named as "formononetin" has been discovered from different plants and has been found involved in antioxidant, anticancer and anti-inflammatory properties (Jiang et al., 2019).

Total antioxidant status value is an indicator of the antioxidant compounds provided by the plant materials. Greater the value of total antioxidant status means higher are the antioxidant compounds in plant extracts (Mohammed et al., 2021). Hussain et al. (2021) made a comparison of phytochemicals present in peel, flesh and seeds of pumpkin (*Cucurbita maxima*), and concluded that pumpkin seeds are excellent sources of total phenolic, flavonoid, carotenoid and mineral contents and due to present of these biologically active components antioxidant status of pumpkin seeds is high as compared to peel and flesh portion. Makni et al. (2011) studied the effect of pumpkin and flax seeds mixtures on alloxan induced diabetic rats and found that diet supplemented with pumpkin seeds exhibited antioxidant activity in rats to control lipid oxidation related diseases. Similar to our results, data was present in the study of Akgul et al. (2022), where ethanolic extracts of plants exhibited strong antioxidant and low oxidant potential, encouraging the use

of plants as natural antioxidant agents. In a study conducted by Erdemli et al. (2018), on the liver cells of rats, it was observed that grape seeds extracts along with low level laser therapy, protected the liver cells and tissues from oxidation.

Total oxidant status of any plant material indicates the whole oxidant compounds produced by plant material due to metabolic reactions occurred in plant cells with variable speed and concentrations due to different environmental and structural factors (Mohammed et al., 2021). Oxidative stress index value is an indication of endogenous oxidant compounds suppressed by endogenous antioxidant agents provided by the plant materials (Dogan et al., 2014).

Table 1. Total antioxidant status, total oxidant status and oxidative stress index of pumpkin seeds ethanolic extracts

TAS, TOS and OSI of pumpkin seeds extracts			
Sample	TAS (mmol/L)	TOS (μ mol/L)	OSI
Pumpkin seeds extracts	4.18 ± 0.36	14.68 ± 0.42	0.35 ± 0.10

Values are presented as means of triplicate results \pm standard deviation

DPPH free radical scavenging activity

Data of free radical scavenging activity of pumpkin seeds ethanolic extracts and ascorbic acid at different concentrations have been presented in Table 2. From the findings it was evident that by increasing the concentration of extracts the DPPH free radical scavenging was found increased as at 0.25mg/mL value of DPPH free radical scavenging activity of pumpkin seeds ethanolic extracts was found 22.96 ± 1.56 and was significantly increased to 56.10 ± 0.90 at 2 mg/mL concentration, while DPPH free radical scavenging activity of ascorbic acid at 0.25 mg/mL was 65.46 ± 0.95 , which was increased to 92.70 ± 0.30 at 2 mg/mL.

Use of traditional herbal remedies as preventive measures and to cure certain diseases are very common in developing and develop countries due to their lesser side effects with fewer complications. The antioxidant potential of a plant or its part depends upon the presence of biological active ingredients capable of suppressing free radicals and reactive oxygen species in living body. Recent advances in medicinal sciences have revealed that peels and seeds of plants have antioxidant potential (Pandey et al., 2019). DPPH free radical scavenging activity of pumpkin seeds 80% methanolic extracts was found 16.56 mg AAE/100 g, in a study conducted by Hussain et al. (2021), in which a comparison of antioxidant and antimicrobial activities of pumpkin peel, flesh and seeds was made. Asif et al. (2017) made research to investigate antioxidant status of different parts of pumpkin, by DPPH free radical scavenging activity of different types of extracts and concluded that pumpkins are capable of promising antioxidant activities. Dissanayake et al. (2018) used DPPH free radical scavenging assay to check the antioxidant potential of pumpkin different fractions by using three different solvents and came on result that all types of solvents provided good results for DPPH free radical scavenging activity of different pumpkin parts. Supportive results were also found in the study of Singh et al. (2016), in which different fruits of *Cucurbitaceae*

family were analyzed for their antioxidant potential by using different solvents using DPPH free radical scavenging analysis and both methanolic and ethanolic extracts exhibited prominent antioxidant activities. Ginkgetin, a natural, non-toxic component from the class of flavones have been found in plants, and has been reported involved to combat cancer progression by arresting the free radicals and stimulating autophagy (Adnan et al., 2020).

Tyan et al. (2018) determined antioxidant activity of three different plants extracts of *Cucurbitaceae* family by using DPPH free radical scavenging method and concluded that by increasing the concentration of extracts dose the free radical scavenging activity was increased. They suggested that pumpkin seeds due to their antioxidant potential could reduce the risk of oxidation related diseases such as cardiovascular diseases and cancer. Hussain et al. (2021) made a comparison of phytochemicals present in peel, flesh and seeds of pumpkin (*C. maxima*), and concluded that pumpkin seeds are excellent sources of total phenolic, flavonoid, carotenoid and mineral contents and these bio-actives are capable of scavenging free radicals. Phenolic compounds such as flavonoids, alkaloids, saponins and steroids present in pumpkin seeds have been reported good antioxidant agents by scavenging the free radicals in living bodies (Mala and Kurian, 2016). Results supporting to our study were found in the research work of Akgul et al. (2022), when DPPH free radical scavenging activity of medicinal plants was compared with ascorbic acid at different concentrations and data obtained revealed that plants ethanolic extracts have potential of scavenging free radicals.

Table 2. DPPH free radical scavenging activity of pumpkin seeds extracts in comparison with ascorbic acid at different concentrations

DPPH free radical scavenging activity of pumpkin seeds extracts		
Concentration mg/mL	Ascorbic acid (%)	EtOH (%)
0.25	65.46±0.95	22.96±1.56
0.5	86.90±0.80	36.60±1.10
1	89.18±0.45	43.58±1.40
2	92.70±0.30	56.10±0.90

Values are presented as means of triplicate results ± standard deviation

Conclusion

Pumpkin is one among the most nutritious vegetable also used as a medicinal food due to its pharmacological potential. Consumption of pumpkin and its seeds have a very long history since ancient times to cure different remedies. Pumpkin seeds extracts TAS was found 4.18±0.36 and TOS 14.68±0.42, whereas value of OSI was measured 0.35±0.10. DPPH free radical scavenging activity was observed increasing with increasing the concentration and maximum value 56.10±0.90% was observed at 2 mg/mL concentration. Pumpkin seeds are excellent source of phytochemicals responsible for strong antioxidant potential, a future prospect for pharma foods industry.

Recommendations

Pumpkin is a versatile vegetable known for its excellent nutritional profile loaded with phytochemicals promoting human health in different ways. Modern and innovative technologies could be implemented to utilize pumpkin in different ways to extract bioactives from pumpkin for development of nutraceuticals and functional foods. Waste streams of pumpkin like peels and seeds can be utilized in efficient ways to develop powders and extracts and their incorporation in bakery, beverages and pharma food industry.

Acknowledgements

The authors are grateful to the Institute of Food Science and Nutrition, University of Sargodha, Pakistan and Institute of Chemistry, University of Sargodha, Pakistan for providing the necessary facilities for conduct of this research work.

Conflict Of Interest

The authors have declared no conflict of interest for this article.

Funding

This research work has not received any funding in any form.

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